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FUSARIUM STRAIN DEVELOPMENT AND SELECTION FOR ENHANCEMENT OF ETHANOL PRODUCTION

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ABSTRACT

Research data obtained at Argonne National Laboratory indicates that selected *Fusarium* strains isolated from natural habitats are potential decomposers and fermenters of biomass. The amount of ethanol produced is comparable to that yielded by other potential microorganisms and, moreover, *Fusarium* strains can ferment xylose (pentoses) while other microbes cannot. Preliminary mutagenesis studies on *Fusarium* isolates indicates that potential mutants can be developed which are capable of hydrolyzing more cellulosics in a shorter time as well as fermenting monosugars to ethanol at higher rates than their parental wild strains. Therefore, new studies were initiated to further enhance the ethanol production via *Fusarium* genetic manipulation. In particular, the aim of this task is to develop superior *Fusarium* strains capable of fermenting monosaccharides (specifically xylose) to ethanol, and able to tolerate higher ethanol concentrations than selected wild strains. Experimental work on hyphal fusions of selected *Fusarium* strains with the purpose of exploiting heterokaryosis and parasexuality for the development of new superior strains has been initiated. Bibliographic information related to *Fusarium* genetics and ethanol fermentation has been studied and a summary is presented.

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INTRODUCTION

It has been estimated that about 30% of the dry content of most crop residues is made of hemicellulose (appearing mostly in the cell walls) [Krull and Inglet, 1980]. Chemical or enzymatic hydrolysis of hemicelluloses produces a mixture of hexoses and pentoses [Gong, et. al., 1981; Lodics and Gong, 1984]. Xylan, one of the major constituents of hemicelluloses upon enzymatic or acid hydrolysis produces xylose which can account for up to 11% of the total monosaccharides of crop residues. In recent years, a considerable research interest has focused on the enzymatic hydrolysis of xylose to produce ethanol with the aim to make the overall biomass fermentation to ethanol an economically sound technology.

Selected strains of both fungal and bacterial species have been tested for their ability to ferment xylose and have shown encouraging efficiency. Furthermore, several techniques have been applied to further enhance ethanol yields from enzymatic hydrolysis of xylose, which have mainly focused on the (a) optimization of fermentation parameters, (b) modification of metabolic pathways, and (c) on the genetic manipulation to develop novel and more potential strains.

Work at Argonne National Laboratory has focused on the use of selected *Fusarium* strains to decompose biomass cellulosics and ferment both pentoses and hexoses to ethanol. Through these efforts more than 3,500 *Fusarium* isolates were obtained from natural substrata, and a few mutant strains were developed via UV-irradiation of selected wild strains. All of them were tested for their potential to hydrolyze cellulose, and nearly all the *Fusarium* isolates showed cellulolytic activity. Tests for extracellular production of cellulase showed an enzyme production ranging from 0.2 to 0.5 IU/mL for wild strains, and up to 1.2 IU/mL for selected strains (including some mutants) in a fermentation of 1% cellulose after 14 days.

During this research the ability of *Fusaria* to produce ethanol from D-glucose and D-xylose was also investigated. All tested *Fusarium* strains were able to ferment glucose and xylose to some extent. Selected strains achieved a conversion of the monosugars to ethanol approximately 83% of the theoretical. Additional efforts indicated that *Fusarium* strains tested for ethanol tolerance were capable of growth and ethanol production in up to 5% ethanol.

The main purpose of the previous work was to produce ethanol via fungal decomposition and fermentation of biomass. Specific objectives were to isolate *Fusarium* strains that effectively saccharify and ferment biomass to ethanol, optimize the parameters that could maximize the ethanol production, and explore the manipulation of key parameters that could increase and accelerate the yield of the needed enzymes. Recently, new work was initiated to further enhance the ethanol production by means of developing new superior *Fusarium* strains which can more efficiently ferment monosugars (specifically xylose) than selected parental wild strains.

THE ASEXUAL BEHAVIOR OF FUSARIA

The asexual stages (imperfect or conidial) of certain ascomycetous (sexual, perfect, ascigerous, or perithecial stages) genera of fungi including *Nectria*, *Calonectria*, *Micronectriella*, and *Giberella*, are classified under the genus *Fusarium* which produces in a specific way uniquely characteristic macroconidia [Booth, 1971].

The asexual stages of the *Fusarium* species are most prevalent and omnipresent in nature. They are represented by two types of conidia: the microconidia which are unicellular or bicellular and have one nucleus per cell; and the macroconidia (conspicuously characteristic for each species) which are multicellular and, again, carry one nucleus per cell. All the nuclei per conidium are genetically identical since they are mitotic progeny of vegetative reproduction [Dickinson, 1932; Buxton, 1954; El-Ani, 1968]. The genetic analysis of the asexual stages of *Fusaria* present difficulties which have recently been mitigated with the discovery of the sexual stages (in most species) with which some genetic work is being conducted.

On the other hand, the sexual stages of *Fusarium* species are represented by the ascospores (of *Nectria*, *Calonectria*, *Micronectriella*, and *Giberella*) which are unicellular, bicellular, or even multicellular. Indications are that these spores have also one nucleus per cell.

The nuclear status of the vegetative hyphal cells of *Fusaria* has not been well determined. In most cases the old cells are uninucleate, while younger cells and the hyphal tips are in several occasions multinucleate [Dickinson, 1932; Koening and Howard, 1962; Hoffman, 1964; Aist and Williams 1972].

Research has indicated that there is a major difference in mitotic nuclear division between *Fusarium* (and similar genera) and other fungi and higher organisms. Work on ultrastructure and time course of mitosis in *Fusarium oxysporum* has shown that mitosis starts with the disappearance of nucleolus followed by chromosome condensation and deep staining, while the nuclear membrane remains unbroken [Aist and Wilson, 1968; Aist and Williams, 1972]. The authors could not observe a true metaphase plate but were able to identify a spindle made of microfibrils connecting the chromosomes with the centriole, and speculated that the microfibril-centriole connection helps chromosome separation during anaphase and the migration of the daughter nuclei.

Studies on meiosis in *Hypomyces solani f. sp. cucurbitae* (= *Nectria haematococca*), the sexual stage of *Fusarium solani*, indicated that all the meiosis phases were well defined like in other organisms [Hirsh, 1949; El-Ani, 1956]. The number of chromosomes for several sexual species of *Fusarium* has been determined to be n = 4, 6 or 7 [Hirsh, et. al., 1949; El-Ani, 1954 and 1956, Howson, et. al., 1963]. *Fusarium oxysporum* (of yet unknown sexual stage) was shown to contain 4 chromosomes [Aist and Williams, 1972]. Through scanty research on DNA content of *Fusarium* species, it has been found that the DNA content is similar to that contained by other fungi [Kumari, et. al., 1975].

During the asexual life of *Fusaria* (and similar fungi) genetic alterations may happen through either heterokaryosis or parasexuality. In heterokaryosis two genetically disimilar vegetative cells (hyphae and/or germ tubes) fuse and their cytoplasm is intermingled but their nuclei remain intact. On the other hand, after heterokaryosis occurs the parental nuclei in an undetermined time recombine and form heterozygous diploids which through a non-meiotic mechanism produce the haploid stage, therefore, following a parasexual pathway to create new strains.

Since 1932, work has indicated that hyphal tip fusions occur in *Fusarium* [Dickinson, 1932], as well as between germ tubes [Buxton, 1954]. Additional research pointed out that fusions happen mostly between old hyphal cells which are uninucleate and not between hyphal tips which are usually multinucleate [Hoffman, 1964, 1966, 1967, 1968]. Several investigators noted that certain factors like succinic acid [Robinson, 1972] temperature [Dickinson, 1932], and UV-irradiation [Buxton, 1956] stimulated or suppressed hyphal fusions in *Fusaria*.

Morphological variants have been obtained by cutting out and culturing fused cells of *Fusaria*, which behaved like nuclear mutants [Dickinson, 1932] and indicated that their parental cells were heterokaryons [Buxton, 1962].

Hoffman [1964, 1966, 1967, 1968] after extensive genetic work with *Fusaria* concluded that isolated sectors in his cultures were haploid recombinants through parasexuality, but was unable to detect heterokarya and diploids and, therefore, considered them very unstable. Heterokaryosis and parasexuality have been demonstrated to occur with the aid of pairing and complementary auxotrophs of *F. vasinfectum* [Ahmed and Shumagasundaram, 1972] and *F. oxysporum f. sp. lycopersici* [Sanchez et. al. 1976]. Researchers have isolated diploids from *F. oxysporum f. sp. pisi* that were so unstable that their detection was inferred from the recovery of haploids. This event supported the occurrence of parasexuality with heterkaryons [Tuveson and Garber, 1959, 1961]. In other fungi the unstable diploids have been maintained with the aid of hyphal tip transfers and incubation at high temperatures [Puhalla et. al., 1974].

Although the production and recovery of *Fusarium* recombinants from pairings of complementary auxotrophs indicated convincingly that heterokaryosis occurs, the nuclear status and the mechanism of heterokaryosis has not been clearly elucidated. It is possible that the transient condition of heterokaryons is an obstacle for such elucidation. However, the creation and recovery of *Fusarium* recombinant haploids is very supportive of the occurrence of parasexuality. It is very probable that parasexuality in *Fusaria* follows the same steps as those occurring in *Aspergillus* [Pontecorvo, 1956] and *Verticullium albo-atrum* [Hastie, 1967] that is, heterokaryon nuclei unite to form a heterozygous diploid nucleus in which a somatic recombination happens and produces haploids. A complete characterization of heterokaryosis and parasexuality in *Fusaria* is lacking.

Recent developments in the genetics of fungi and bacteria could be applicable to investigate heterokaryosis and parasexuality in *Fusaria* and characterize their heterokaryons, diploids, heterozygotes, and haploids as well as elucidate the mechanism of their formation.

It has been noted that several *Fusarium* isolates during their subculturing show a morpholoigical variability, that is, change their mycelial morphology and in some instances produce sectors of growth different in appearance than the rest of the culture. These variants are mostly stable and can be maintained by monoconidial transfer or hyphal tip culture. It has been suggested that this variation is rare in nature [Miller, 1946]. Studies on the relationship of nutrient requirements as well as on the effects of chemicals contained in the growth medium on morphological variability of *Fusaria* have demonstrated the stimulability of these factors towards generation of variants [Brown, 1926; Miller et. al., 1947; Prasad, 1949; Georgopoulos, 1963].

Studies have indicated that the sector variants suppress the parental strain [Miller, 1945] and the type (micro-, or macroconidium) and source (single conidiophore or sporodichium) of spore initiating the culture affect the frequency of variant production [Miller, 1945; Cormack, 1951]. It has been found that maintainance of *Fusarium* stock cultures on soil extract agar or sterile soil, and under low temperature, as well as transfer by a microconidium or single hyphal tip reduce the variation.

The genetic cause for the spontaneous variability in *Fusarium* is still unexplored. Heterokaryon analysis has shown the nuclear genetic basis of the variants [Dickinson, 1932; Buxton, 1954; Dimock, 1937; Snyder et. al., 1975]. Although there are indications that these variants are cytoplasmic mutants (characteristic of some fungi) [Singh, 1973], no work has determined the nuclear or cytoplasmic variation in *Fusaria*.

Ultraviolet light irradiation has shown to greatly enhance the generation of morphological variants in *Fusarium* species [Miller, 1945; Buxton, 1956; Imshenetski and Ulianowa, 1962; MacNeil and Sabanayagam, 1968; Antonopoulos and Wene, 1984]. Several chemicals have also been tested which induce variation in *Fusaria*, like nitrous acid, ethylmethanosulfonate and nitrosoguanidine [Bouhot, 1970; Sanchez et. al., 1976; Van Etten and Kolmark, 1977], zinc salts [Dimock, 1936], benomyl [Dassenoy and Meyer, 1973] fernasan [Abdalla, 1975], and other pesticides.

FUSARIUM XYLOSE FERMENTATION AND INITIAL EFFORTS FOR DEVELOPING SUPERIOR STRAINS

Fusarium strains have been shown to ferment monosaccharides (both pentoses and hexoses) to ethanol in appreciable amounts [Birkinshaw et. al, 1931; White and Willaman, 1928; Anderson et. al., 1933; Antonopoulos and Wene, 1985; Batter and Wilke, 1977; Chiang et. al., 1982; Cochrane, 1956; Du Preez, et. al., 1983; Gibbs, et. al., 1954; Heath et. al.; 1956; Jeffries, 1985; Jeffries et. al.; 1985; Letcher and Willaman, 1926; Rosenberg et. al., 1980; Suiko and Enari, 1981].

Our research efforts in the past focused on studying the ability of selected *Fusarium* strains to ferment D-glucose and D-xylose to ethanol. Nearly all *Fusaria* tested were able to ferment both sugars to some extent. Additional efforts identified strains which yielded about 4.3 mg/mL ethanol in 48 hours in 1% glucose and xylose. During this work it was established that anaerobic conditions were not favorable for *Fusarium* cell growth and rapid ethanol production. Semi-aerobic conditions shortened fermentation times and increased cell-mass production.

As it has been reported [Antonopoulos and Wene, 1985], with a combination of strain selection and control of fermentor conditions several of the newly isolated *Fusaria* have produced up to 4.3 mg/mL ethanol from 1% D-xylose and up to 8.4 mg/mL from 2% D-xylose in 48 hours. In comparing our results with selected *Fusarium* strains and those obtained by others working recently with the yeasts *Pachysolen tannophilus* and *Candida shehatea* [Sliniger et. al. 1982; Du Preez et. al. 1983; Jeffries, 1985; Jeffries et. al., 1985] some conclusions can be derived. *Fusarium* strains are not as productive per unit cell mass as some of the yeasts, but they are as efficient in terms of yield. However, anaerobic *Fusarium* fermentations are more productive per unit cell mass, but the fermentation times are longer. The specific fermentation rate may be compensated for by recycling cell mass. For *Fusaria* to be competitive with some of these yeasts the

volumetric fermentation rate must be increased. One way to increase this is to decrease losses of ethanol due to respiration. Some of our studies showed that glucose addition to xylose fermentations produced higher ethanol yields.

A recent article [Jeffries, 1985] listed certain requirements which must be fullfilled to make a pentose fermentation to ethanol a successful commercial process. The suggested requirements are: (a) an ethanol yield of at least 0.40 g/g of pentose sugars; (b) ethanol production of at least 50 g/liter; (c) fermentation time of less than 36 hours, and (d) complete utilization of the pentose sugars.

Fusaria isolated and tested in our laboratory fullfill two of the above requirements. Ethanol yields from selected *Fusarium* strains typically range from 0.40 to 0.43 g of ethanol per gram of pentose sugar and the pentose sugar is over 99% utilized. In order for a fermentation to yield 50 g/liter of ethanol the fermentation medium must contain between 100 and 125 g/liter of pentose sugar. The *Fusarium* strains that we have isolated in this laboratory are not able to completely utilize pentose sugar at this concentration. The maximum ethanol production which has been achieved from 100 g/liter of xylose is 29 g/liter in a batch fermentation. In a fermentation conducted in a batch fed mode the ethanol yield increased to 34 g/liter.

A fermentation time of less than 36 hours was the next suggested parameter necessary for successful ethanol production from pentose sugar. *Fusarium* isolates ferment 2% xylose to 8.4 mg/mL ethanol within 48 hours. Increasing concentrations of xylose require longer fermentation times. Fermentation of 4% xylose to a yield of 16 mg/mL requires 96 hours. Fermentation of 5% xylose in a batch fed mode to 20 mg/mL can also be accomplished in the same amount of time. If economic goals could be reached with 2% ethanol streams then these *Fusarium* isolates would be candidates for the process if the fermentation times could be reduced. This could most likely be accomplished with the isolation and development of superior strains and a refinement of fermentation techniques. This has been the area where our research has concentrated. Studying the parameters that affect the rate of ethanol production and looking for new *Fusarium* strains that grow and produce ethanol at higher rates.

Based on literature information, initial research steps have been taken to explore and use the heterokaryotic and parasexual pathways of *Fusaria* with the aim to develop new superior strains for the enhancement of ethanol production from monosugars, specifically xylose.

During the past, the use of UV-irradiation developed *Fusarium* strains which were superior to the wild strains in fermenting cellulose, glucose and xylose. UV-mutants were obtained by irradiation of uninucleate microconidia. After repeated subculturing the mutant strains did not retain their higher levels of cellulase enzyme production. This decrease in activity may be similar to other problems encountered in retaining cultural characteristics of *Fusaria* during continuous transfer and storage. Spontaneous genetic change is often cited as a reason for the loss of certain characteristics in culture. In some cultures, sectoring of the growth pattern has been observed, with the loss of aerial mycelium and changes in pigmentation. It was possible that the production of variants was stimulated by the medium components and conditions of growth.

Current experiments are using *Fusarium* strains ANL-99A, ANL-22, ANL-22760, ANL-8, ANL-8-82481, and ANL-325 which have shown exceptional ability to ferment glucose and xylose, as parental material to develop new strains. Two different strains have been inoculated on one plate and their adjacent mycelia are under microscopic observation for hyphal fusions and nuclear behavior. So far, no substantial results have been obtained due to the recent initiation of this project.

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REFERENCES CITED

Abdalla, M. H. 1975 "Sectoring of Fungal Colonies Induced by Low Concentrations of Fernasan," Mycopathologia, 56:39-40.

Ahamed, N. M. M., and Shanmugasundaram, E. R. B. 1972. "Biochemical and Genetical Studies in Host-Parasite Relationships. Effect of Heterokaryosis on the Degree of Virulence of Nutritional Mutants of *Fusarium vasinfectum*," Phytopathol. Z. 75:349-359.

Aist, J. R., and Williams, P. H. 1972 "Ultrastructure and Time Course of Mitosis in the Fungus *Fusarium oxysporum*," J. Cell Biol. 55:368-389.

Aist, J. R., and Wilson, C. L. 1968. "Interpretation of Nuclear Division Figures in Vegetative Hyphae of Fungi," Phytopathology 58:876-877.

Anderson, A. K., Everitt, E. L., and Adams, P. D. 1933. "The Carbon Metabolism of *Fusarium oxysporum* on Glucose," J. Agric. Research 46:473-82.

Antonopoulos, A. A., and Wene, E. G. 1985. "Biomass Fermentation to Ethanol by Selected *Fusarium Strains*," DOE/SERI Biochemical Conversion Program Semi-Annual Review Meeting, Denver, Colorado, pp. 307-328.

Batter, T. R., and Wilke, C. R. 1977. "Study of the Fermentation of Xylose to Ethanol by *Fusarium oxysporum*," Lawrence Berkeley Lab., Univ. of California at Berkeley Report, 99 p.

Birkinshaw, J. H. 1931. "Quantitative Examination by the Carbon Balance Sheet Method of the Types of Products Formed from Glucose by Species of *Fusarium*," In "Studies in the Biochemistry of Microorganisms", H. Raistrick (ed.) Phil. Trans. (London) 222(467B) 93-8.

Booth, C. 1971 The Genus *Fusarium*. Commonw. Mycol. Inst., Kew, Surrey, England. 237.

Bouhot, D. 1970. "Variations Induities du Pouvoir Pathogene chez *Fusarium oxysporum f. sp. melonis*," Ann. Acad. Sci Fenn. A., IV. 1968:25-27.

Brown, W. 1926. "Studies in the Genus *Fusarium*, IV. On the Occurrence of Saltations," Ann. Bot. 40:223-243.

Buxton, E. W. 1954. "Heterokaryosis and Variability in *Fusarium oxysporum f. gladioli (Snyder & Hansen)*," J. Gen. Microbiol. 10:71-84.

Buxton, E. W. 1956. " Heterokaryosis and Parasexual Recombination in Pathogenic Strains of *Fusarium oxysporum*," J. Gen. Microbiol. 15:133-139.

Buxton, E. W. 1962. "Parasexual Recombination in the Banana-Wilt *Fusarium*," Trans. Brit. Mycol. Soc. 45:274-279.

Chiang, L. C, et. al. 1982. "Ethanol Production from Pentoses by Immobilized Microorganisms," Enzyme Microb. Technol. 4:93-95.

Cochrane, V. W. 1956. "The Anaerobic Dissimilation of Glucose by *Fusarium lini*," Mycologia 48:1-12.

Cormack, M. W. 1951. "Variation in the Cultural Characteristics and Pathogenicity of *Fusarium avenaceum and F. arthrosporioides*," Can. J. Bot. 29:32-45.

Dassenoy, B. amd J. A. Meyer. 1973. "Mutagenic Effects of Benomyl on *Fusarium oxysporum*," Mut. Res. 21:119-120.

Dickinson, S. 1932. The Nature of Saltation in *Fusarium* and *Helminthosporium*. Minn. Agric. Exp. Stn. Bull. 88. 42 p.

Dimock, A. W. 1936. "Variation in a Species of *Fusarium* Induced by High Concentrations of Zinc Salts," Zentralbl. Bacteriol., Abt. 2, 95:341-347.

Dimock, A. W. 1937. "Hybridization Studies on a Zinc-Induced Variant of *Hypomyces ipomoeae*, Mycologia 29:273-285.

DuPreez, J. C., and Van der Walt, J. P. 1983. "Fermentation of D-Xylose by a Strain of *Candida shehatae*," Biotechnoil. Lett. 5:357-362.

El-Ani, A. S. 1956. " Ascus Development and Nuclear Behavior in *Mypomyces solani f. cucurbitae*," Amer. J. Bot. 43:769-778.

El-Ani, A. S. 1968. "The Cytogenetics of the Conidium in *Microsporum gypseum* and of Pleomorphism and the Dual Phenomenon in Fungi," Mycologia 60:999-1015.

Georgopoulos, S. G. 1963. "Tolerance to Chlorinated Nitrobenzenes in *Hypomyces solani f. cucurbitae* and Its Mode of Inheritance," Phytopathology 53:1086-1093.

Gibbs, M., et. al. 1954. "Fermentation of D-Glucose-1-C14 by *Fusarium lini Boley*," Arch. Biochem. Biophys. 50:237-242.

Gong, C.-S., Chen, L. F., Flickinger, M. C., and Tsao, G. T. 1981. "Conversion of Hemicellulose Carbohydrates," Biochem. Eng. 20:93-118.

Hastie, A. C. 1967. "Mitotic Recombination in Conidiophores of *Verticillium albo-atrum*," Nature 214:249-252.

Heath, E. C., Nasser, D., and Kiffler, H. 1956. "Biochemistry of Filamentous Fungi, III, Alternative Routes for the Breakdown of Glucose by *Fusarium lini*, Arch. Biochem Biophys. 64:80-87.

Hirsch, H. E. "The Cytogenetics of Sex in *Hypomyces solani f. cucurbitacea*," Amer. J. Bot. 36:113-121.

Hirsh. H. E., Snyder, W. C., and Hansen, H. N. 1949. "Chromosome Numbers in the *Hypocreaceae*, " Mycologia 41:411-415.

Hoffman, G. M. 1964. "Untersuchungen uber die Kernverhaltnisse bei *Fusarium oxysporum f. callistephi*," Arch. Mikrobiol. 49:51-63.

Hoffman, G. M. 1966. "Untersuchungen uber die Heterokaryoseildung und den Parasexualcyclus bei *Fusarium oxysporum*. I. Anastomosenbildung im Mycel und Kernverhaltnisse bei der Conidienentwicklung," Arch Mikrobiol. 53:336-347.

Hoffman, G. M. 1967. "Untersuchungen uber die Heterokaryosebildung und den Parasecualcyclus bei *Fusarium oxysporum*. III. Pararungsversuche mit Auxotrophen Mutanten von *Fusarium oxysporum* f. callistephi," Arch Mikrobiol. 56:40-59.

Hoffman, G. M. 1968. Kernverhaltnisse bei Pflanzenpathogenen Imperfekten Pilzen, Insbesondere Arten der Gattung *Fusarium*," Zentralbl. Backteriol. Abt.2, 122:405-519.

Howson, W. T., Mcginnis, R. C., and Gordon, W. L. 1963. "Cytological Studies on the Perfect Stages of Some Species of *Fusarium*," Can. J. Genet. Cytol. 5:60-64.

Imshenetski, A. A., and Ulianowa. 1962. "Experimental Variability in *Fusarium moniliforme Sheld.* Leading to the Formation of Gibberellins, " Nature 195:62-63.

Jeffries, T. W. 1985. "Emerging Technology for Fermenting D-Xylose, "Trends in Biotechnol. 3:208-212.

Jeffries, T. W., Fady, J. H., and Lightfoot, E. N. 1985. "Effect of Glucose Supplements on the Fermentation of Xylose by *Pachysolen tannophilus*," Biotechn. Bioeng. 27:171-176.

Koening, R., and Howard, F. L. 1962 "Nuclear Division and Septum Formation in Hyphal Tips of *Fusarium oxysporum*." Amer. J. Bot. 49:666 (Abstr.).

Krull, L. H. and Inglet, G. I. 1980. "Analysis of Neutral Carbohydrates in Argicultural Residues by Gas-Liquid Chromatography," J. Agric. Food Chem. 28:917-919.

Kumari, L., Cecallone, J. R., and Meyer, J. A. 1975. "Deoxyrobonucleic Acid Metabolism and Nuclear Division During Spore Germination in *Fusarium oxysporum*," J. Gen. Microbiol. 88:245-252.

Letcher, H., and Willaman, J. J. 1926. "Biochemistry of Plant Diseases, VIII, Alcoholic Fermentation of *Fusarium lini*," Phytopathology 16:941-949.

Lodics, T. A., and Gong, C. -S. 1984. "Isolation of Sugarcane Bagasse Hydrolyzate-Tolerant Yeast Mutants by Continuous Selection," Biotechnmol. Bioeng. Symp. 14:303-307.

MacNeil, B. H., and Sabanayagam, J. V. 1968. "The Induction of Dodine Tolerance in *Fusarium oxysporum f. melonis*: a Technique Applicable to the Study of Bionomics of Soil-Borne Fungal Pathogens," Can. J. Microbiol. 14:1262-1263.

Miller, J. J. 1945. "Studies on the *Fusarium* of Muskmelon Wilt. I. Pathogenic and Cultural Studies with Particular Reference to the Cause and Nature of Variation in the Causal Organism," Can J. Res. C. 23:16-43.

Miller, J J. 1946. "Cultural and Taxonomic Studies on Certain *Fusaria*. I. Mutation in Culture," Can J. Res. C. 24:188-212.

Miller, J. J., Kock, L. W., and Hildebrand, A. A. 1947. "A Comparison of Cultural Methods for the Maintenance of Certain Economic Fungi," Sci. Agric. 27:74-80.

Pontecorvo, G. 1956. "The Parasexual Cycle in Fungi," Annu. Rev. Microbiol. 10:393-400.

Prasad, N. 1949. "Variability of the Curcurbit Root-Rot Fungus, *Fusarium (Hypomyces) solani f. cucurbitae*," Phytopathologg 39:133-141.

Puhalla, J. E., and Mayfield, J. E. 1974. "The Mechanism of Heterokaryotic Growth in *Verticillium dahliae*," Genetics 76:411-422.

Robinson, P. M. 1972. "Isolation and Characterization of a Branching Factor Produced by *Fusarium oxysporum*," Trans. Brit. Mycol. Soc. 59:320-322.

Rosenberg, S. L., et. al. 1980. "Hemicellulose Utilization for Ethanol Production," Lawrence Berkeley Lab., University of California at Berkeley.

Sanchez, L. E. Leary, I. V. and Endo, R. M. 1976. "Heterokaryosis in *Fusarium oxysporum f. sp. lycopersici*," J. Gen. Microbiol. 93:219-226.

Schneider, R. 1958. "Untersuchungen uber Variabilitat und Taxonomie von *Fusarium avenaceum (Fr.) sacc.*," Phytopathol. Z. 32:95-126.

Singh, U. P. 1973. "Effect of Acriflavine on UV-induced Mutants of *Fusarium* Species," Mycopathol. Mycol. Appl. 50:183-193.

Sliniger, P. J., et. al. 1982. "Conversion of D-Xylose to Ethanol by the Yeast *Pachysolen tannophilus*," Biotechnol. Bioeng. 24: 371-84.

Snyder, W. C. Georgopoulos, S. G., Webster, R. K., and Smith, S. N. 1975. "Sexuality and Genetic Behavior in the Fungus *Hypomyces (Fusarium) solani f. sp. cucurbitae*," Hilgardia 43:161-185.

Suiko, M. L., and Enari, T. M. 1981. "The Production of Ethanol from D-Glucose and D-Xylose by Different *Fusarium* Strains," Biotechnol. Lett. 3:723-728.

Tuveson, R. W., and Garber, E. D. 1959. "Genetics of Phytopathogenic Fungi II. The Parasexual Cycle in *Fusarium oxysporum f. pisi*," Bot. Gaz. 121;74-80.

Tuveson, R. W., and Garber, E. D. 1961. "Genetics of Phytopathogenic Fungi IV. Experimentally Induced Alterations in Nuclear Ratios of Heterokaryons of *Fusarium oxysporum f. pisi*," Genetics 46:485-492.

Van Etten, H. D., and Kolkmark, H. G. 1977. "Modifying the Growth Habit of the Filamentous Fungus *Fusarium solani* to Facilitate Replica Plating Procedures," Can. J. Bot. 55:848-851.

White, M. G., and Willaman, J. J. 1928. "Biochemistry of Plant Diseases, X, Fermentation of Pentoses by *Fusarium lini*," Biochem. J. 22:583-591.